REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1997	Annual (1 Oct 96 - 30 Sep 97)

4. TITLE AND SUBTITLE
Linkage of Molecular and Epidemiological Breast Cancer Investigations with Treatment Data: A Specialized Registry

5. FUNDING NUMBERS
DAMD17-94-J-4114

6. AUTHOR(S)
O. Ross McIntyre, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Dartmouth College
Hanover, New Hampshire 03755-3580

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

During the first ten months of the third year of this project, the Cancer and Leukemia Group B (CALGB) entered 102 breast cancer patients into the Linked Registry according to procedures specified in CALGB protocol 9484. The telephone interviews have gone smoothly, and the data entry is proceeding without difficulty. The flow of samples from these patients has been most satisfactory and there have been no problems with preparation of DNA from them, or with the handling of serum samples and tissue blocks. Although accrual has increased substantially as a result of actions taken during year three, it is still less than originally anticipated for this project. The Steering Committee has met and reviewed alternate projects for which a smaller than anticipated Registry will be of value. The Registry will begin to supply samples for these and certain of the projects originally proposed during year four of the project.

14. SUBJECT TERMS Humans, Clinical Trials, Anatomical Samples, Breast Cancer, Genetics, Epidemiology, Etiology, Prevention

15. NUMBER OF PAGES 32

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

AD_____

Grant Number DAMD17-94-J-4114

TITLE: Linkage of Molecular and Epidemiological Breast Cancer Investigations with Treatment Data: A Specialized Registry

PRINCIPAL INVESTIGATOR: O. Ross McIntyre, M.D.

CONTRACTING ORGANIZATION: Dartmouth College
Hanover, New Hampshire 03755-3580

REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

NA Where copyrighted material is quoted, permission has been obtained to use such material.

NA Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

ORM Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

NA In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

ORM For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

NA In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

NA not applicable

O Ross [illegible] 9/15/97

PI - Signature Date

Table of Contents

Progress Report
Contract DAMD 17-94-J- 4114 from U.S. Army Research and Materiel Command

October 1, 1996-September 30, 1997

Prepared by O. Ross McIntrye, M.D. Principal Investigator

I. INTRODUCTION:

A. Nature of the problem:

The use of adjuvant chemotherapy following local treatment of the tumor has clearly benefited many patients with breast cancer[1]. On the other hand, adjuvant chemotherapy carries with it a number of potential risks including secondary malignancies. Thus, it would be desirable to give adjuvant therapy only to the subgroup of women with breast cancer who are most likely to have a recurrence. Although clinical findings are useful in assigning prognosis [2,3], these alone are imperfect measures and there is hope that additional tests, such as the detection of certain somatic mutations in the tumor, will prove helpful in guiding the decision as to who should and who should not receive adjuvant chemotherapy. These considerations have now been formalized in the language describing such testing and a distinction between prognostic factors (which forecast clinical outcome) and predictive factors (which predict response and influence selection of specific forms of therapy) has been offered.[4]

In addition to our ability to detect a number of somatic mutations that may predict the risk of recurrence, it is now possible to identify those individuals who carry mutations in the BRCA 1 or BRCA 2 gene in their germline[5,6,7,8,9,10]. It is anticipated that additional genes conferring an increased risk of breast cancer upon their carriers will be identified. The presence or absence of susceptibility alleles in the germline may influence not only risk of occurrence of breast cancer but also the response to treatment and other outcomes in these patients. Knowledge that such genes are present may predict the likelihood of a second primary in women who have already been diagnosed with breast cancer, and may assist in guiding prevention efforts in other members of the family who carry the gene.

The investigators who are participating in this project will test a number of hypotheses that were described in our original application. In addition, the project will provide resources for other studies, four of which have been described in previous project reports and several others will be considered by the Steering Committee later this year.

Because interactions of erbB-2 and p53 with type of adjuvant therapy received have already been observed (see next section), it is important that assays for putative prognostic factors be performed on well-characterized groups of patients receiving adjuvant chemotherapy according to standardized protocols. The registry being created with support from this grant is quite different from usual population-based registry concepts. Instead, it may be viewed as a library in which clinical information on groups of uniformly staged and treated patients is located within a structure that also contains patient personal, family, and environmental exposure history, specimens from patients, and data from molecular and other laboratory studies. In contrast to a population-based registry, it offers an internally cohesive group of patients with well-defined disease, treatment and follow-up. It is possible to draw scientifically valid conclusions from this group by looking for interactions between treatment and factors such as genomic susceptibility and acquired somatic alterations.[11]

In cohorts of patients treated on our protocols, endpoints such as time to recurrence, site of first recurrence, percentage of planned adjuvant therapy received, and detailed initial staging information are systematically recorded. Moreover, there is an opportunity to collect additional information (dietary, smoking, and exposure history) from such patients that may be useful in predicting the likelihood of a germline mutation or other factors that may interact with treatment and prognosis.

The identification in a patient or family member of a breast cancer patient of a heritable gene conferring an increased risk of breast cancer carries with it economic and psychosocial risks[12] in addition to the possibility that the gene is not causally related to the cancer in that patient[13]. We will be able to assess the impact of determining genomic susceptibility on individuals *most in need* of this type of information. The creation of the linked registry supported by this grant offers the opportunity for the patients and those involved in the laboratory to be joined in the pursuit of new knowledge. It is important that this pursuit be conducted in a manner offering the least psychological stress and the greatest protection from adverse social and economic consequences to those who participate. Collection of detailed information at the time of entry to the study relating to this as well as other areas will allow hypotheses concerning this aspect of the study to be tested.

B. Background and Previous Work

In order to show the value of our linked-registry we offer the example that follows. We emphasize that this is an early example of the type of success we hope to achieve. The work that produced these results followed the successful integration of effort by a number of individuals, funded by a variety of sources including NCI grants to the CALGB, R01 and SPORE grants held by certain of the investigators, and by a small foundation grant to the CALGB.

Example: In 1989, the CALGB activated protocol 8869 with Hyman Muss, M.D., Bowman Gray School of Medicine, as study chair. The goal of this study was to pursue possible relationships between S phase and ploidy in breast cancer

specimens, as determined by flow cytometry techniques, with clinical outcome in patients treated on our adjuvant protocol 8541. The protocol provided for collection of fixed tissue on a random sample of patients entered on the treatment study. As 8869 progressed, and as techniques were perfected for the immunohistochemical determination of erbB-2 and P53 on paraffin embedded specimens, the protocol was amended so that Ann Thor, M.D., of Massachusetts General Hospital, could apply these tests to the specimens. In addition, molecular assessment of these tissues by Edison Liu, M.D., of the University of North Carolina was added at that time.

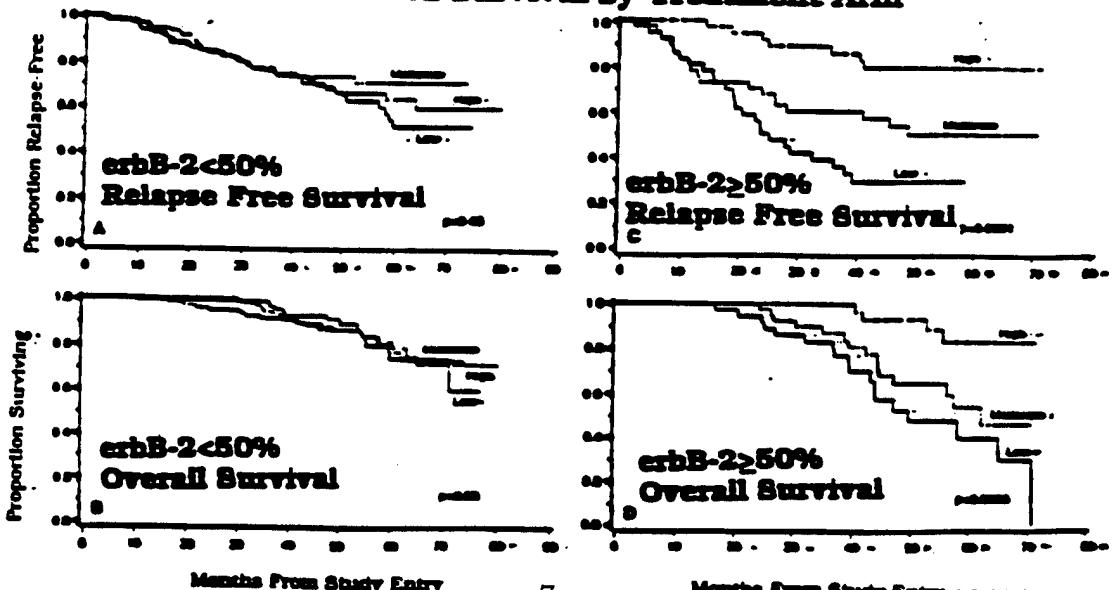
The treatment protocol, 8541, tested three CAF (cyclophosphamide, doxorubicin, and 5-fluorouracil) adjuvant regimens for which the dose schedule and dose intensity are shown in Table 1. Patients receiving the more dose intense regimens had significantly longer disease-free and overall survival than patients receiving the lower dose regimen.[14, 15]

Table 1
Dose and Dose-Rates

Arm	I	II	III
Dose Rate mg/M2/week			
Cyclophosphamide	150	100	75
Doxorubicin	15	10	7.5
5-Fluorouracil	300	200	150
Cumulative Dose mg/M2			
Cyclophosphamide	2400	2400	1200
Doxorubicin	240	240	120
5-Fluorouracil	4800	4800	2400

When these treatment results are combined with studies of S-phase, P53 and erbB-2, an unexpected highly significant finding emerged.[16,17] The effect was most dramatic

Figure 1
erbB-2 and Survival by Treatment Arm



for erbB-2 which is shown in Figure 1, although similar results occurred when P53 overexpression[18,19] or S-phase were analyzed.

As shown, the patients whose tumors overexpressed erbB-2 had a significantly longer disease-free and overall survival than those whose tumors did not overexpress erbB-2. There was no significant difference in disease-free or overall survival with any of the three treatments for those patients whose tumors did not overexpress erbB-2. These findings indicate that the benefit of intensive adjuvant therapy with this combination is limited to a subgroup of patients. From the clinical data we know that the group receiving the more intensive treatments fared better, but without the integrated laboratory data, we would have no indication that the patients with the best outcomes represented two populations, one which did, and the other which did not, benefit from the more intensive treatments.

As stated above, 8869 originally collected specimens on a randomly selected sample of all patients on 8541. Committing almost all of the very limited non-NCI funds available to the CALGB, we immediately set about to collect all of the remaining blocks available from patients on this study. During the two years the analysis of tests for S phase, P53 and erbB-2 which were performed on this second set of specimens has been going forward and a publication providing further evidence for the hypothesis that doxorubicin dose intensity interacts with P53 and erbB-2 overexpression is under review by he Journal of The National Cancer Institute.

Conclusion from the Example: The issue of dose intensity, time to failure or death, and erbB-2 (or P53) overexpression would not have been raised if the laboratory study had not been conducted on tissue samples of similarly staged patients receiving randomly assigned, defined therapeutic regimens.

The rapidly building capabilities for this type of study and the excitement attending the initial success of combining laboratory and clinical information on our patients has led to several meetings of investigators. At these meetings there has been vigorous discussion of the opportunities for new projects as well as the need to develop new resources to serve CALGB as well as other investigators. The infrastructure category of the Army BAA offered an ideal mechanism to advance our studies and to assist other investigators in the field.

C. Purpose and Hypothesis:

We are using well-established methods within the CALGB and new procedures developed with support of this project to create a specialized registry which links molecular and epidemiological data with data from uniformly staged breast cancer patients receiving defined therapy. This registry of data, tumor tissue, and other specimens will enhance the research of 30 to 50 peer reviewed and funded investigators during the course of the project. It is intended that the level of quality control as well as the comprehensiveness of the registry will make it an unparalleled resource for investigators pursuing the relationship between tumor genetics, tumor biology and the prevention and treatment of breast cancer.

D. Methods of Approach - Specific Technical Objectives:

This project creates a linked-registry based upon the capabilities of CALGB to rapidly enroll large numbers of well-characterized incident breast cancer patients to its treatment trials. It takes advantage of a unique opportunity to link data on the biology of breast cancer with information on uniformly staged patients who receive defined treatments. Since TNM staging defines rather broad categories[20], especially in stage II breast cancer, we anticipate that an exploration of the sources of heterogeneity with newly developed markers will advance our understanding of the disease.

This registry is used for studies on epidemiological and molecular characteristics that influence the outcome for breast cancer patients. The registry will provide information critical to the design of future chemo-prevention studies, the interaction of treatment with factors that govern disease progression and metastasis, and will be instrumental in guiding the design of future adjuvant treatment trials.

Specific technical objectives are as follows:

a. To modify questionnaires currently in use by CALGB investigators at the University of North Carolina, University of Minnesota and NIEHS to collect key family history and exposure data in a self-completed questionnaire.

b. To establish review procedures and criteria for selecting patients with a family cancer history for further study. Criteria will include, but are not limited to, having one or more first-degree relatives with breast cancer or having 2 or more relatives with breast, ovarian, or colon cancer.

c. To develop a telephone interview with patients identified for further study that will expand on the screening data collected, obtain information that will facilitate validation of cancer reported, and locate selected siblings for inclusion in the database.

d. To collect fixed breast tissue from patients and germ-line DNA, plasma, and urine from the same patients.

e. To review and confirm the histopathological diagnosis of breast cancer on submitted tissue.

f. To integrate information about specimen receipt, specimen availability, and laboratory testing results with the CALGB data base and to prioritize use of this information.

g. To modify the CALGB data base and data handling procedures at the CALGB Statistical Center at Duke University, so as to efficiently capture and record information from the registry, and to furnish it to users.

h. To augment resources at CALGB institutions in order to procure the above described information and specimens.

II. BODY OF THE APPLICATION

A. Description of the Methods:

In contrast to laboratory-based investigations, the linked registry employs new and existing committees of the CALGB and new resources created by the registry to collect specimens as well as epidemiologic and psychosocial information. It provides a mechanism to integrate registry data with clinical information derived from CALGB clinical trials. Specimens and information from the registry are to be used by laboratory-based investigators, epidemiologists, and others to test various hypotheses bearing on breast cancer cause, risk, progression, response to treatment, as well as to determine the psychosocial impact of this testing.

This project is based at Dartmouth Medical School where Dr. McIntyre, the Principal Investigator, serves as the James Carroll Professor of Oncology, Emeritus. Subcontracts from Dartmouth provide support for activities at the University of North Carolina (DNA extraction and epidemiology), Georgetown University (Lombardi Cancer Center - serum and urine bank), Roswell Park Cancer Institute (tissue sectioning, tissue banking and pathology review), the University of Chicago (communication, protocol editing, and regulatory compliance) and Duke University (statistics and data management). Where possible, efficiencies are achieved by using methods of communication, data submission, protocol editing, meeting arrangements, etc., that have been developed for the CALGB.

The Principal Investigator, Dr. McIntyre, is assisted in the management of the project by three committees:

Table 2
Linked Registry Steering Committee

Name	CALGB position	Institution
O. Ross McIntyre, M.D.	Committee Chair	Dartmouth
Robert Millikan, DVM, Ph.D	Co-PI	U. North Carolina
Maurice Barcos, M.D.	Pathology Com. Chm	Roswell Park
Donald Berry, Ph.D.	Statistician	Duke Univ.
Ira Bleiweiss, M.D	Pathologist	Mt. Sinai Hospital
Daniel Hayes, M.D.	Cor. Sci. Chm.	Georgetown Univ.
Larry Norton, M.D.	Br. Com. Chm	MSKCC
Barbara Smith, M.D.	Surgery	Mass. General Hospital
Lynn Dressler, M.A.	Cor. Sci. Com.	U. North Carolina
Dale Sandler, Ph.D.	Epi. Com. Chm.	NIEHS
Debra Collyar	Patient Advocate	External Member
Susan Moore	Patient Advocate	External Member

This interdisciplinary committee is responsible for overseeing the conduct of the project, assisting with the integration of projects that will use the registry so as to insure the greatest productivity from it, and setting priorities for use of the resource.

Epidemiology Resource Committee: This committee is responsible for the design of the data collection instruments employed by the linked registry. It is also responsible for the review and prioritization of projects requesting use of linked registry data. The committee is chaired by Dale Sandler, Ph.D, Chief, Environmental and Molecular Epidemiology Branch, NIEHS, and Chair of the CALGB Epidemiology Committee.

Table 3
Epidemiology Resource Committee

Name	CALGB position	Institution
Dale Sandler, Ph.D.	Chair	NIEHS
Robert Millikan, DVM, Ph.D.	Co P.I.	U. North Carolina
Beth Newman	Epidemiologist	U. North Carolina
Stephanie London MD, Ph.D.	Epidemiologist	Univ. So. Cal.
Matthew Longnecker, MD, ScD	Epidemiologist	UCLA
Thomas Sellers, Ph.D.	Epidemiologist	U. Minnesota
Fred Li, M.D.	Epidemiologist	Dana Farber
Donald Berry, Ph.D.	Statistician	Duke University
Virginia Ernster Ph.D.	Epidemiologist	U. of California S.F.
Barbara Smith, M.D.	Surgeon	Mass. General Hospital

In brief, the committee has developed and implemented procedures to collect family cancer history, reproductive and hormone use history, and other exposure information from all breast cancer patients enrolled in CALGB treatment trials. Tumor tissue and germ-line DNA is collected from breast cancer patients as described below.

Breast cancer patients who are registered to CALGB treatment trials are informed by CALGB clinical research associates and nurse oncologists about this project at those CALGB institutions where CALGB 9484 has been activated. Patients are offered the opportunity to participate in a treatment companion protocol, CALGB 9484 that provides for the gathering of epidemiological data and collection of specimens. During 1996 CALGB protocol 9484 was amended so as to improve patient accrual. The revised protocol has been reviewed and approved by the Army Research and Materiel Command. The amended protocol was issued to CALGB institutions on October 15, 1996. The rationale for the changes embodied in the amended protocol are given in section B, below. The methods described in this section are those specified in the amended protocol and differ somewhat from procedures described in last year's progress report.

Breast cancer patients who give their informed consent for treatment on selected CALGB breast cancer protocols are asked to return a self-completed questionnaire

and to give permission for submission of their biopsy specimen as well as blood and urine samples. In addition we ask the patient's permission to conduct studies of germline DNA on cells obtained from a blood sample. The consent form for these procedures is integrated with that of the treatment protocol and administered at the same time. We follow all consent procedures mandated by Department of Defense regulations and IRBs at participating institutions. The self-completed questionnaire was appended to the progress report for year 2 of the project.

Questionnaires are collected by the institutional clinical research associates who submit them to the CALGB Data Management Center at Duke. There, they are examined for completeness, checked for errors, and the data entered in the CALGB data base.

On the basis of information from the self-completed questionnaire, the investigators at UNC, under the direction of Dr. Millikan, categorize the patients into three groups:

a. Patients with any first or second degree relative with breast or ovarian cancer.
b. Patients aged <50 years with no family history.
c. Patients aged ≥ 50 years with no family history.

All patients in groups a and b, and a random sample of group c, above are contacted by the telephone interviewer. Consenting patients are then queried in the telephone interview. The questionnaire administered by telephone was furnished in Appendix 2 of the progress report for year 2 of the project.

Our previous experience has shown that it is necessary to conduct telephone or in-person interviews to verify and complete family histories and exposure history. Because recall bias is introduced in self-reports of breast cancer occurrence in first degree relatives[21] a carefully administered interview to confirm the self-reporting is indicated. Telephone interviews work as well as in-person interviews for this purpose.[22,23]

We have found an 85% participation rate in our telephone interview inquiring about risk factors for leukemia and this is carried out while these acutely ill patients are hospitalized. While the response rate for the self-completed questionnaires is often lower than that for telephone or in-person interviews, we anticipate a high rate of return of the initial questionnaire because institutional data managers are responsible for retrieving the completed forms. We have used telephone interviews with great success not only in the environmental exposure studies in leukemia patients but also in the long-term follow-up of patients with successfully treated Hodgkin's disease.[24,25,26]

Tissue Resource Coordinating Committee: The Breast Tissue Coordinating Committee, Chaired by Lynn Dressler, University of North Carolina, serves to coordinate the systematic collection and archiving of breast tissue, germ-line DNA, serum, plasma, and urine.

Table 4
Tissue Resource Coordinating Committee

Name	CALGB position	Institution
Lynn Dressler M.A.	Chair	U. of North Carolina
Robert Millikan, DVM, Ph.D.	Co P.I.	U. of North Carolina
Maurice Barcos, M.D.	Pathology	Roswell Park
Joe Gray, Ph.D.	Genetics	U. of California S.F.
Daniel Hayes, M.D.	Oncology	Georgetown
Hyman Muss, M.D.	Oncology	University of Vermont
Donald Berry, Ph.D	Statistician	Duke University

1. Fixed tissue:

When the patient signs an informed consent to participate in CALGB 9484 institutional clinical research associates (CRAs) arrange for submission of tissue blocks by contacting the coordinating pathologist at a CALGB main member or affiliate institution. Paraffin blocks and sample submission forms are received at the CALGB Pathology Coordinating Office directed by Dr. Maurice Barcos at Roswell Park Cancer Institute. There, they are logged into the CALGB data base and histologic sections are made. Four micron slides from these submissions are sent to Dr. Fred Koerner at the Massachusetts General Hospital who reviews them for accuracy of diagnosis, and delineates areas on the slides containing homogeneous malignant tissue. These slides are returned, the blocks trimmed, if necessary, to yield 4 FISH (immunohistochemistry) and 10 micron sections (PCR, FISH) of homogeneous tumor, as well as non-malignant breast tissue. At least 30 sections are removed: 20, 4 micron sections for immunohistochemistry/FISH/ISH assays and 10, 10 micron sections for molecular based assays requiring extracted DNA. At three levels, sections are taken and stained and examined to ensure representative tissue is being distributed for all assays. We ask for permission to retain the blocks for future sectioning and store them at 4° C. If this is not granted, we prepare sections as described above, as determined by the amount of tissue available in the block. Prior to the return of the blocks to the submitting institution we prepare additional sections.

2. DNA procurement:

Somatic DNA: From the specimens collected as described above, individual investigators prepare somatic DNA according to their established laboratory procedures.

Germline DNA: EDTA anticoagulated peripheral blood is collected and shipped to the UNC DNA extraction laboratory overnight for lymphocyte separation and DNA extraction. Lymphocyte DNA is prepared using the ABI DNA extractor and the DNA stored at -70°C. Yield and quality of extracted DNA are monitored on an ongoing basis.

Quality control/quality assurance/sample distribution for DNA extraction:

The CALGB/RPCI Pathology Coordinating Office cuts and mounts a series of 10 micron sections on uncoated slides from each block according to their routine procedures. These procedures incorporate careful quality control and quality assurance parameters, including changing the microtome blade between each block to prevent contamination of DNA on the blade surface, cleaning the waterbath surface between each block, and wearing gloves to process blocks. As part of the routine processing procedure at the RPCI Pathology bank, sections for H & E staining are cut immediately preceding and after those cut for molecular (10 micron section) and immunohistochemical (4 micron) assays. The CALGB Pathology Coordinating office reviews all H & E sections to ensure that representative and sufficient tumor tissue is present throughout all sections cut for assay. In addition, to enrich for tumor tissue, tumor-rich versus tumor-poor areas are marked on the corresponding H & E section(s). For DNA processing, the corresponding H & E section will be superimposed on the unstained 10 micron sections and the circled region of tumor rich areas will be isolated and scraped into an Eppendorf tube by the technician in the UNC tissue bank. DNA lysates will be prepared as described below from each tumor tissue. DNA lysates are stored at 4 degrees centigrade for short term storage and at -85 degrees centigrade for long term banking. DNA lysates are stored in vials and multiple aliquots of processed DNA are prepared. As indicated above, DNA processing occurs in a clean area: a special room where only tissue and DNA processing is allowed to prevent DNA contamination, a major problem in PCR based studies. Distribution of samples is defined in the CALGB protocol and is rigorously monitored both in house and through the CALGB DMC Tissue tracking system. A protocol is only developed once the study has received appropriate review and approval from the Solid Tumor Correlative Science Committee and Central Tissue Bank Committee.

Protocol for DNA Extraction from Tissue Sections:

Formalin fixed paraffin embedded tissue sections (1-5 depending on cellularity and size of tumor area) are gently scraped from uncoated glass slides (uncoated slides facilitates the scraping process, although tissue can be scraped from coated slides as well) with a 200 ul micropipet tip into a 1.5 microfuge tube. In a fume hood, 500 ul of xylene is added to each tube and the tubes are thoroughly mixed. After a 5 minute centrifugation at 1200 rpm, the supernatant is discarded into a xylene waste container, and the pellet is extracted twice with 500 ul of autoclaved 95% ethanol. the pellets are dried for 2 hours or more in a vacuum dessicator before addition of 200 ul lysis buffer containing Proteinase K and overnight incubation at 58° C. On the following day the Proteinase K is inactivated by a 10 minute incubation at 95° C. Any remaining debris is removed by a 10 minute centrifugation in the microfuge, and the supernatant is ready to use as a template source for a variety of molecular analyses.

3. Collection of plasma, serum and urine:

Plasma samples are collected into EDTA-containing collection tubes. After separation from the cellular component, the plasma is aliquoted to a freezing tube, labeled, and frozen at -20°C at the participating institution. These samples are batched and when

several tubes have been collected, they are shipped on dry ice overnight to Georgetown University (Lombardi Cancer Center), where they are catalogued, kept at 4°C for short term storage and -70°C for long term storage. Frozen urine is shipped in batches to the Georgetown for processing and analysis.

4. **Training of data managers:**

On a regular basis, not less than once a year, a portion of the CALGB CRAs workshop is devoted to instruction of the proper methods of obtaining and shipping the above specimens.

5. **Receipt of Specimens:**

Centers receiving specimens will electronically report to the CALGB data base the receipt and condition of the specimen using standard CALGB procedures.

6. **Tracking of Patient Specimen Submission:**

The CALGB data management system tracks patients who are entered on CALGB protocols and plans to implement a system soon that will generate reminders to institutions that have entered patients on treatment protocols if the required specimens have not been received at the appropriate office or lab in a timely manner.

Use of the data from the Linked Registry: All uses for the information in the linked registry will be described in formal protocols that define the objectives, methodology, and statistical assumptions. These must be reviewed and approved by the Steering Committee. Letters are sent to the users setting out the agreement under which they use the registry. These were included in Appendix 3 of the progress report for year 2 of the project. Written proposals from the scientific community are considered if they do not compete with approved projects already underway, and are prioritized with respect to anticipated amount of tissue or resources consumed vs. the likely yield of important information. In assigning this priority to scientists who are not CALGB members we use the same scale that will be used for projects developed by CALGB members. In all cases emphasis is placed upon the level of innovation and the track-record of the investigator with respect to peer review and publications. We plan to deliberately include projects, however, from young investigators without a track record, if they are endorsed by knowledgeable mentors and are innovative.

The availability of the Linked Registry is publicized through usual channels of scientific communication. In addition, the CALGB newsletter that is sent to many investigators outside the CALGB will be used as will news releases to "The Cancer Letter", and similar publications. Eventually, information about the CALGB Linked Registry will be available at the CALGB World Wide Web site.

B. Progress in Year 3.

Introduction: The progress report for year two submitted one year ago mentioned that patient accrual to this study was far less than anticipated. It also described steps that were being taken to address this problem. During year one, it appeared that the availability of genetic counseling within CALGB institutions, a resource needed if the results of testing for familial cancer gene testing were to be made available to study subjects, was the principal stumbling block to adoption of the protocol at many CALGB institutions. We recognized this need during the design phases of the project and described it in our application. Beginning in the second year of the project we instituted a program intended to provide extensive training in the necessary genetic counseling skills. At that time, we anticipated that results of genetic testing would be available during year 4 of the project.

Despite the progress of the training program, the number of institutions approving the study remained far less than anticipated and it became clear during the second year of support that other aspects of the project also caused concern at our institutions. During this time, several articles appeared in the scientific and lay press calling attention to the potential risks involved in familial gene studies. Although these publications recommended that such testing should occur in the context of research trials of the sort this project represents, Institutional Review Boards (IRBs) were not willing to approve testing for familial cancer genes in the context of cooperative clinical trials. We were asked to assist in providing information addressing these concerns and amended the protocol to address the principal issues these committees raised about the project. Nevertheless, the accrual remained much lower than anticipated in the third year of the project.

Apart from accrual, all other goals set for year three of the project have been met. The impact of the low accrual upon the types of research this project can support has been assessed during year three of the project and various alternative research projects are briefly described in the final section of this report. The pilot testing of the questionnaire has been completed, specimen submission has gone smoothly and a review of the forms submitted on patients entered to date has revealed no problems.

Patient Accrual and Revision of CALGB Protocol 9484 to Improve Accrual: Table 5 below shows accrual to the Linked Registry (CALGB Protocol 9484) and to concurrent CALGB breast cancer treatment trials.

Table 5
Accrual to CALGB 9484 vs Samples Received on Concurrent CALGB Treatment Studies (cut off date 7/1/97)

Registrations to CALGB 9484	133
Registrations to CALGB Treatment Protocols	1201
Tissue blocks received CALGB 9344 (Adjuvant Rx)	695
9484 samples received from patients on 9344	59
Total samples on CALGB 9484 for gene studies	127
Tissue blocks received on 9484	96

Inspection of table 5 indicates that only a small fraction of patients placed upon concurrent treatment trials entered CALGB 9484. For instance tissue blocks were received on 695 patients entered onto CALGB 9344, the recently completed adjuvant treatment study for patients with stage II breast cancer. Of these, only 59 patients were registered to CALGB 9484. The submission of tissue blocks on a large fraction of patients placed upon treatment trials demonstrates that the linked registry concept is technically feasible if IRB approval of the protocol occurs at all CALGB institutions and if the paperwork required for patient entry to the protocol can be simplified at the institutional level. (This is why the CALGB has attempted to combine entry to the treatment protocol with entry to the Registry (9484) by combining the consent forms for both studies, see below.)

Most importantly, nearly all patients who have agreed to participate in CALGB 9484 have also agreed to provide a specimen for familial gene studies (127 of the 133 patients entered). Since provision of such a specimen is not a requirement for participating in 9484, this indicates that most patients who are informed about the study and agree to participate are willing to have familial gene studies performed.

Protocol amendment:

In an attempt to improve accrual the original protocol was amended in 1996. The revised protocol contained the following changes:

1. Patients were no longer given the option of receiving the results of familial gene studies performed on their specimens. Such testing had become commercially available thereby obviating ethical issues that had led us to offer to provide test information, if desired by the patient, in the original study design. (See previous progress reports for the background leading to these decisions.) As a result of this change:

a) there was no longer a requirement that the institution must have a genetic counseling program in place for the study patients,

b) confidentiality issues posed by the return of results of research genetic tests to the institution were avoided, and

c) since the research results would be located in the CALGB data base at Duke University rather than in the records of about 200 institutions, the process of obtaining a Certificate of Confidentiality from the Department of Health and Human Services would be simplified.

Accrual to the amended protocol:

Despite these changes, the number of participating institutions and the number of patients entered into the study increased more slowly than anticipated during the second half of 1996 and the first half of 1997. With the closure of CALGB protocol 9344 in April, 1997, the major breast cancer adjuvant study led by CALGB and the source of the majority of new CALGB breast cancer patients, the Steering Committee met June 2, 1997 in Chicago to consider further steps that could be taken to improve accrual.

Wording required in consent forms: The protocol covering the activities of the Registry, CALGB 9484, had been amended at the request of the investigators and institutions so that the consent form for investigational treatment and that for participation in the Registry were combined. This resulted in a significant increase in efficiency at our institutions. However, the Office for Protection from Research Risks (OPRR) of the NIH found that language required by the Army concerning the donation of specimens was "exculpatory" and indicated that it would not approve the combined model consent form. The Steering Committee recommended that the Principal Investigator and his colleagues attempt to resolve this issue. Several phone calls and letters to the parties involved failed to eliminate the impasse and at present CALGB has activated its new adjuvant treatment protocol with two separate consent forms in order to satisfy a review by the parties funding the treatment and Registry functions respectively. As a result there will be various inefficiencies at the institutional level and it is possible that the accrual to 9484 will decline somewhat from its current level of 10 per month.

We have suggested to the Army that the language concerning the donation of the tissue and fluids be changed to the following: "**In signing this consent form I donate the blood, urine and tissue samples that will be obtained from me to the Cancer and Leukemia Group B for the purposes of the research described in this consent form. The project staff, supported by a contract from the U.S. Army Research and Materiel Command to Dartmouth College, will use these samples exclusively for the research described above.**" We understand that this wording is not perceived as exculpatory by OPRR.

In addition, it is reasonable to assign a negligible risk to having a blood sample drawn for our project at the same time that a blood sample is being obtained for routine blood work. Completing a telephone administered questionnaire is also a negligible risk. For this reason we have proposed dropping the phrase required by the Army stating that the contractor will "support the cost of medical care should illness from participating in this protocol occur". IRBs do not understand that Dartmouth College is the contractor, not their institution, and that Dartmouth has accepted this risk as the contractor. We are told that the consent forms are referred to institutional counsels who, lacking background in this matter, suggest non-approval. **We recommend**

dropping this statement from the consent forms for our 200 institutions, since it is not relevant to the type of research being supported.

Acceptance of these changes by the Army will likely result in improved accrual.

Other Actions to Improve Patient Accrual:

1. *Requirement that information on research subjects be maintained for 75 years by the federal government:* We were informed during the review of the revised consent form for CALGB 9484 that since this project is viewed by the Army Research and Materiel Command as posing minimal risk to the patients there was no need to submit long term follow-up information on participants to the federal government. The consent form has been changed to reflect this.

2. *Problems with submission of tissue blocks to our tissue repository in a state where state regulations had been interpreted as prohibiting this activity.* A ruling from the New York State Department of Health that the CALGB Pathology Coordinating Office may act as a repository for such specimens was issued August 26, 1996. This eliminates the problem in that state and sets a precedent for other states where this issue could be raised.

3. *Certificate of Confidentiality:* A Certificate of Confidentiality covering this project was obtained from the Department of Health and Human Services in 1996.

Progress toward meeting Specific Technical Objectives:

a. **To modify questionnaires currently in use by CALGB investigators at the University of North Carolina, University of Minnesota and NIEHS to collect key family history and exposure data in a self-completed questionnaire.**

The self completed patient questionnaire contains items from the above sources and additional input from the team led by Dr. Fred Li at the Dana Farber Cancer Institute has occurred so as to yield a questionnaire that meets the broad needs of investigators. Under the leadership of Dr. Millikan and Ms. Cirrincione, a draft self completed questionnaire was developed that addressed the needs of the patients and was capable of being interfaced with the CALGB data management system. Pilot testing in CALGB institutions during the early spring of 1995 revealed several problems which were corrected in a further draft that was tested in April. The final version is incorporated in CALGB protocol 9484 which was mailed to CALGB institutions on May 15, 1995 for activation.

The telephone interviews with study participants have gone well and with excellent patient cooperation. Because accrual has been less than anticipated during this period, telephone interviews have been carried out on all patients rather than the originally planned sample.

Because participants in the amended protocol will not receive information concerning familial gene status, study participants will no longer consist of two groups: those who wish and those who do not wish to know their status with respect to familial cancer genes. Thus, portions of the original questionnaire dealing with the topic of the choice to receive information on gene carrier status will be no longer be relevant. The questionnaire has been modified accordingly and pilot testing of these modifications has been successful.

b. To establish review procedures and criteria for selecting patients with a family cancer history for further study. Criteria will include, but are not limited to, having one or more first-degree relatives with breast cancer or having two or more relatives with breast, ovarian, or colon cancer.

This technical objective was changed during our budget negotiations prior to activation of the project given the budget limitations. We will not allocate those with a family history of colon cancer into the group for the telephone interview. By so doing we will

(i) enrich for BRCA1 and BRCA2 and potentially AT families, rather than diluting our efforts with potential mismatch repair families,

(ii) avoid overlap with a proposed colon cancer susceptibility study supported by other funding

(iii) allow us to focus (as we should) on breast cancer screening and treatment issues, even though colon cancer is an important disease.

The purpose of developing the selection criteria is to yield a pool of individuals with a family history of breast cancer who will participate in an intensive telephone interview. This hour-long interview was developed with input, not only by investigators from this project, but in concert with others who have grants from the U.S. Army Research and Materiel Command to support related investigations. In addition, a control group of individuals without a family history of breast cancer who are under treatment on CALGB breast cancer protocols is included, as noted above, for comparison purposes.

c. To develop a telephone interview with patients identified for further study that will expand on the screening data collected, obtain information that will facilitate validation of cancer reported, and locate selected siblings for

inclusion in the database. The study will obtain exposure information from affected and unaffected first-degree relatives of patients with a family history of cancer.

The telephone interviews are proceeding well and there are no problems with this aspect of the study.

As noted above, the interviewing of family members was eliminated from the project prior to study activation as a result of the need to reduce the budget.

d. To collect fixed breast tissue from patients and germ-line DNA, plasma, and urine on the above patients and family members.

CALGB 9484, covering the submission of tissues and specimens listed above, was mailed to CALGB institutions on May 15, 1995. As of September, 1997 the protocol has been approved by the IRBs in 78 of 215 CALGB institutions.

e. To review and confirm the histopathological diagnosis of breast cancer on submitted tissue.

This activity is proceeding without any problems.

Infrastructure and Policy Development:

Overview:

The Pathology Coordinating Office has developed an integrated coordination and communication network through the Tissue Resource Coordinating Committee for the systematic collection, archiving, surveillance, quality control and quality assurance for the acquisition and processing of the fixed, paraffin tissue blocks for this study. The appointment of a tissue bank coordinator, who also serves as the CALGB Vice Chair and Tissue Bank Coordinator for solid tumor correlative science studies will facilitate and expedite this integration, interfacing with database management, maintaining appropriate quality control and quality assurance procedures for the storage and processing of tissues, and developing policies to respond to institutional pathology concerns about tissue banking. In addition, we have identified coordinating/contact pathologists at each of our main and affiliate institutions to expedite case accessioning of paraffin blocks and to establish a network of communication for responding to mutual concerns and problems that may develop during the course of the study (additional efforts to integrate pathology participation are discussed section 3). The following sections describe pathology policy that we have developed for tissue banking (see Section A-1 and Appendix 6 of the progress report for year 2), and detailed procedures for processing to ensure quality control and quality assurance as

well as steps taken to avoid depletion of the block (Appendix 7 of the progress report for year 2).

Pathology policy development for tissue banking:

Although tissue acquisition for this study commenced October, 1995, the Pathology Coordinating Office has had experience collecting blocks as a mandatory requirement for four breast cancer clinical trials in the CALGB. Because of varying certification and licensing requirements placed at the federal, state and professional society level concerning retention of blocks by institutional surgical pathology laboratories, it is not always clear whether all or simply representative tissue blocks are required to remain on file by a pathology laboratory. Some hospital policies prohibit release of an entire block for storage, but will allow cut sections to be stored. Many hospitals are willing to release blocks if they can be assured of accessibility to representative material for any future medical-legal need. In order to address these concerns, and offer alternatives for those hospitals whose policies prohibit release of an entire block for storage, we have developed a Tissue Bank policy for this study (Appendix 6 of the progress report for year 2).

Quality control and quality assurance of tissue blocks/sections:

Several precautions are taken to ensure that appropriate processing is performed to accommodate a variety of laboratory uses. High quality sections that are representative of the histopathologic diagnosis of breast cancer are required. For example, to reduce possible DNA contamination for molecular assays the following precautions are taken: gloves are worn by the histotechnician, the disposable blade is wiped down with 10% bleach, followed by 70% alcohol between each block unless a new blade is used; the water bath surface is cleaned between each block, clean forceps are used for each block. In addition, all thick, 10 micron sections cut for molecular assays are placed on uncoated slides (to facilitate scraping) and are stored at 4 degrees. All intact blocks are stored at 4 degrees to minimize antigen deterioration. Thin sections cut for immunohistochemistry are stored at a minimum of 4 degrees (preferably -70°C) and are placed on coated slides (to avoid tissue detachment during assay). H & E sections are cut at different levels throughout the block to ensure that representative tissue is being used for a particular assay. These procedures also address the steps to be taken when minimal tissue is available from the block. This ensures that tissue will not be exhausted in these blocks. A detailed procedure for processing of tissue sections for molecular, immunohistochemical and flow cytometric assays was offered in Appendix 7 of the progress report for year 2.

Efforts to Integrate Pathologist Participation in this Study:

The institutional pathologist is a critical link for accessing representative tissue for laboratory studies. However, in the cooperative group setting, the pathologist has often not participated in breast cancer studies except in the

submission of tumor locks to the Pathology Coordinating Office. In 1997, Dr. Ira Bleiweiss of Mount Sinai Hospital was named as the coordinating pathologist for breast cancer studies and was named to the Steering Committee for the project. In an effort to enhance integration of pathologists into the cooperative research process for breast cancer clinical trials and correlative science studies, Pathology Workshops are held at CALGB meetings to disseminate information regarding breast cancer studies, to discuss the active role that pathologists can play in these studies and provide a forum for problem resolution with respect to accession and tissue banking.

f. To integrate information about specimen receipt, specimen availability, and laboratory testing results with the CALGB data base and to prioritize use of this information.

This activity is a major goal for year 4 of this project.

g. To modify the CALGB data base and data handling procedures at the CALGB Statistical Center at Duke University, so as to efficiently capture and record information from the registry, and to furnish it to users.

Under the leadership of Ms. Donna Hollis and Gloria Broadwater, the first half of the above objective has been met. As information concerning these studies is gathered, the second portion of this task will be performed, namely the integration of the information with clinical characteristics, response to treatment and other endpoints.

Further thinking about the research design has indicated that a goal of furnishing the database information to users is inappropriate. Instead, the results of laboratory and other investigations will reside in the database and will be accessed by CALGB statisticians in order to address hypotheses offered by all investigators participating with CALGB in this project.

h. To augment resources at CALGB institutions in order to procure the above described information and specimens.

Payments to institutions to cover the costs of selecting or obtaining specimens has begun. Because accrual has been slower than originally anticipated, the cost to the project for reimbursement of institutional expenses has been less than originally budgeted.

III. SUMMARY

A. Conclusions:

1. CALGB Protocol 9484, providing the basis for specimen and data collection for a Linked Breast Cancer Registry, has been amended and approved by the Army Research and Materiel Command for review. Despite the improvement in accrual that has resulted, patient entries to the Registry are still far less than originally anticipated. Possible reasons for this are offered in the text of the progress report. In addition, certain new research initiatives that can be accomplished with the smaller number of patients available within the registry are summarized.

2. Problems concerning the nature and process of informed consent for studies of familial breast cancer genes have been addressed.

3. The procedures used for the telephone interview have been developed, pilot tested, revised and implemented. The responses from the patients to the telephone interview have been gratifyingly positive.

4. The DNA extraction apparatus has been purchased, installed, and is in use at the University of North Carolina, Chapel Hill. The freezer for urine and plasma samples that was purchased at the Dana Farber Cancer Institute, Boston has been moved to Georgetown University where Dr. Hayes, the subcontractor for this portion of the project, has recently assumed a faculty position. Specimens of plasma and urine are now being shipped to that location.

5. Workshops for CALGB data coordinators and genetics counselors were held November 1995 in Dallas, Texas, May 1996 in Miami, Florida, and November 1996 in Pittsburgh. Another workshop is scheduled for June 1998 in Fort Lauderdale, FL.

6. *Changes in Project Staff:*

   Transfer of the CALGB Central Office to the University of Chicago: As mentioned in the report for year 2 of the Registry, on March 31, 1995, Dr. McIntyre's 5 year term as Chairman of CALGB was completed and he was replaced as Group Chair by Dr. Richard Schilsky, Director of the University of Chicago Cancer Research Center, who had been elected to the position. There has been no material effect of the change in the location of the Central Office upon this project. Nor has there been any change in the time-line as a result of the move of the Central Office. There will be no change in the cost of the project.

   Dr. Liu assumed the position of Director of the Division of Clinical Sciences, National Cancer Institute and departed from his previous position at the University of North Carolina, Chapel Hill (UNC) during the summer of 1996. Lynn Dressler, M.A. who has played a major role in the project at that institution has been appointed to take over the responsibility for the subcontract at UNC.

This transition has been very smooth and no problems are anticipated because of Dr. Liu's departure. Dr. Liu will continue to interact with his laboratory at UNC weekly during the next year.

Dr. Daniel Hayes, moved from the Dana Farber Cancer Center, Boston, to the Lombardi Cancer Center at Georgetown University, Washington, D.C. The specimens, laboratory equipment and subcontract supporting his participation in this project have been moved to this new location and there were no problems with this transition.

Dr. Ira Bleiweiss was appointed as coordinating pathologist for breast cancer studies within CALGB and has been named to the Steering Committee for this project. His biosketch is furnished in Appendix 1.

B. Changes Resulting from Experience in Year 3. Problems and Corrective Actions.

The major problem during year 3 has been the continued slower than anticipated accrual to CALGB 9484. The initial version of our protocol was developed as a consensus among those with expertise with regard to regulations concerning use of human subjects and with the input of cancer advocates. Nevertheless, there has been much slower than expected approval of the protocol at our institutions as described above. Although our project was conceived and designed to minimize the risks involved in this kind of research, the appearance of cautionary articles for the lay and scientific community [27] resulted in a changed climate for the institutional review of this project. The protocol has been approved in only 36% of CALGB institutions.

Reassessment of the types of research the Registry will support in view of the lower than anticipated accrual: At the Steering Committee meeting June 2, the Group considered the resources the project will have to offer investigators by the end of the 4 year project. As the Registry was originally conceived some proposals for its use entailed analyses from large numbers of patients (more than 1,000). Other research could be successfully completed with the number of specimens likely to result from the Registry at its current rate of accrual. These were reviewed and additional projects were discussed. Some of the projects under consideration by the Steering Committee are listed below:

1. Case-case (case series) comparison: Risk factors for breast cancer in patients with a family history versus in women without a family history.

2. Prevalence of patients with a positive family history and other characteristics of women in clinical trials. We do not know how representative clinical trial patients are versus breast cancer patients in general in terms of family history and other factors.

3. Evaluate self administered questionnaires: Are they as valid as a more detailed family history obtained via phone interview?

4. Samples are requested to test hypotheses developed by Holland, et al. These investigators, using PCR technology, have reported finding a 660 base pair sequence, unique in the gene bank, for a portion of the envelope gene of the murine mammary tumor virus (MMTV) in 38% of breast cancer specimens from American women. The sequence is not found in other tumors, normal tissues, or tissues other than breast cancer of the affected patients. Archival specimens confirm this finding, with 37% positive using a 250 base pair sequence within the 660 base pairs.[28]

Based on this preliminary data, the investigators hypothesize:

a) Breast cancer in American women associated with the sequences homologous to MMTV is different in its behavior from breast cancer which is negative by PCR.

b) Breast cancer arising during pregnancy or lactation is more likely to be associated with the sequences (80% in the preliminary data).

c) Breast cancer positive for MMTV env-like sequences is likely to occur at an earlier age than negative tumors.

d) Breast cancer specimens are likely to be associated with increased frequency of axillary nodal metastasis.

e) Metastatic axillary lymph nodes containing breast cancer from a primary neoplasm that is positive by PCR would also be positive, and that metastatic axillary lymph nodes of breast cancers negative by PCR will be negative.

5. Polymorphisms in DNA repair genes and response to chemotherapy: Dr. Harvey Mohrenweiser, Human Genome Center, Lawrence Livermore National Laboratory, has proposed a collaboration using the Registry. He has recently identified common variants in genes involved in DNA repair. These variants could provide important prognostic information for patients undergoing adjuvant chemotherapy or radiation therapy. The nucleotide excision repair (NER) pathway removes bulky adducts from DNA, including those produced by a wide array of chemotherapeutic drugs. Deficiencies in NER could increase responsiveness to chemotherapeutic drugs, since effective removal of bifunctional DNA adducts leads to drug resistance[29,30]. A second DNA repair mechanism, the double-strand break (DSB) - recombination pathway, is involved in repair of radiation-induced DNA damage[31]. Patients with suboptimal repair of radiation-induced DNA damage are at increased risk for breast cancer[32,33,34,35,36] and might be at increased risk for recurrence and/or side-effects following radiotherapy. The existence of DNA repair genes with common, low penetrant alleles has been hypothesized[33] but none

have been identified to date. Identification of such genes would be a considerable advance, and could lead to more effective uses of chemotherapy and radiotherapy tailored to the responsiveness of individual patients.

Preliminary data.

Dr. Mohrenweiser has identified several common variants (allele frequencies of 10% or greater) in genes involved in both NER and DSB repair pathways. Variants in NER include alterations in ERRC1, and variants in DSB repair include alterations in XRCC1, XRCC3 and RAD51. The role of these genes in breast cancer has not been examined to date, but NER and DSB repair are likely to be relevant for both etiology and progression of breast cancer. Recently, the C-terminus of BRCA1 was shown to contain a region of homology with XRCC1 and other DNA repair proteins[37] and RAD51 binds to both BRCA1[38] and BRCA2[39].

Proposed project.

We plan to genotype participants in the Specialized Registry for polymorphisms in NER and DSB repair genes. We will investigate two types of interactions:

(i) By combining NER/DSB genotype information with treatment information, we can examine gene - environment interaction. We hypothesize that patients with NER defects will respond better to high dose chemotherapy, and patients with DSB defects will be at increased risk for recurrence following radiotherapy.

(ii) By combining NER/DSB genotype information with assessment of germline alterations in BRCA1 and BRCA2, we can address gene-gene interactions. It is possible that inheritance of rare mutations in BRCA1/2, combined with inheritance of common alterations in NER and/or DSB repair genes, combines to increase or decrease therapeuticresponse.

In this collaboration Dr. Douglas Bell, National Institute for Environmental Health Sciences, will supervise high-throughput genotyping of DNA repair gene variants in samples from patients in the Specialized Registry. As part of previously funded projects in the University of North Carolina Specialized Program of Research Excellence (SPORE) in breast cancer, we are already conducting genotyping of DNA repair variants in Dr. Bell's laboratory, and the specimens from the Specialized Registry will be analyzed at no cost.

Retention of Tissue Blocks: An unexpected number of institutions have requested that the blocks submitted on 9484 be returned immediately after sections have been

taken and have cited various regulatory or legal requirements as the reason for these requests. In order to resolve this problem we have taken two courses of action. We try to convince those making this request that it is reasonable for us to have custody of their blocks as long as we demonstrate that we can return them to the institution within one business day of a request for their return. Second, we maintain paraffin sections at low temperatures in order to preserve antigens. In addition, we indicate, on the slide, the date the sections were cut . We are also conducting time-course experiments to optimize storage conditions for detection by new antibodies as they become available for use. This may stabilize antigens that have been shown to deteriorate in sections maintained at the higher temperature.

V. REFERENCES

[1]Bonadonna, G. and Valagussa P.: Dose response effect of adjuvant chemotherapy in breast cancer. N. Engl. J. Med., 304:10-15, 1981

[2]Fisher, B. Redmond, C. Fisher, EB. et al: The contribution of recent NSABP trials of primary breast cancer therapy to an understanding of tumor biology. Cancer 46:1009, 1980

[3] Ellege RM, Clark GM, Chamness GC, Osborne CK. Tumor biologic factors and breast cancer prognosis among white, Hispanic, and black women in the United States. Jr. Nat. Can. Inst. 86:705-12, 1994

[4] Gasparini G, Pozza F, Harris A. Evaluating the potential usefulness of new prognostic and predictive indicators in node-negative breast cancer patients. J Natl Cancer Inst 85: 1206-19, 1993.

[5] Goldgar DE, Fields P, Lewis CM, Tran TD, Cannon-Albright LA, Ward JH, Swensen J, Skolnick MH. A large kindred with 17q-linked breast and ovarian cancer: genetic, phenotypic, and genealogical analysis. Jr. Nat. Can. Inst. 86:200-9, 1994.

[6] Miki, Y., Swensen, J., Shattuck-Eidens, D., et. al., A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene BRCA1, Science, 266:66-71,1994.

[7] Futreal, P.A., Liu, Q., Shattuck-Eidens, D., et. al., BRCA 1 mutations in primary breast and ovarian carcinoma, Science 266:120-122, 1994.

[8] Miki Y, Swensen JJ, DeHoff BS, Rosteck PR, Skolnick MH, Neuhausen SL. A physical map encompassing GP2B, EPB3, D17S183, D17S78, D17S1183, and D17S1184, Genomics 25:295-7, 1995

[9] Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Mikclem G, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature, 378: 789-92, 1995

[10] Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-lyonnet D, Belanger C, Bell R, Berry S, Bogden R, Chen Q, Davis T, Dumont M, Frye C, Hattier T, Jammulapati S, Janecki T, Jiang P, Kehrer R, Leblanc JF, Goldgar DE, et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nature genetics 12: 333-37, 1996

[11] Rothman, K. *Modern Epidemiology,* pp 95-96, Little Brown, Boston, 1986

[12]Hoskins KF, Stopfer JE, Calzone KA, Merajver SD, Rebbeck TR, Garber JE, Weber BL. Assessment and counseling for women with a family history of breast cancer. A guide for clinicians. Jr. Am. Med. Assn. 273:577-85, 1995

[13] Shatzkin A, Goldstein A, Freedman LS. What does it mean to be a cancer gene carrier? Problems in establishing causality from the molecular genetics of cancer. Jr. Natl. Can. Inst. 87:1126-1130, 1995

[14] Budman DR, Wood W, Henderson IC, Korzun AH, Cooper R, Younger J, Hart RD, Moore A, Ellerton J, Norton L, Ferree C, Colnagelo A, McIntyre OR. Initial findings of CALGB 8541: A dose and dose intensity trial of cyclophosphamide(C), Doxorubicin(A), and 5-fluorouracil(F) as adjuvant treatment of stage II, node +, female breast cancer. Proc Am Soc Clin Onc 11: 51, 1992 .

[15] Wood WC, Budman DR, Korzun AH, Cooper R, Younger J, Hart RD, Moore A, Ellerton J, Norton L, Ferree C, Colnagelo Ballow A, Frei E III, Henderson IC. Dose and dose intensity trial of adjuvant chemotherapy for stage II, node positive breast carcinoma; Initial results of CALGB 8541, New Engl Jr Med. 330:1253-9, 1995

[16] Muss H, Thor A, Kute T, Liu E, Koerner F, Berry D, Cirrincione C. erbB-2 (c-erbB-2; HER-2/neu) and S-phase fraction (SPF) predict response to adjuvant chemotherapy in patients with node positive (N+) breast cancer (BC). Proc Am Soc Clin Onc 12:72, 1993.

[17] Muss HB, Thor A, Berry DA, Kute T, Liu ET, Koerner F, Cirrincione CT, Budman DR, Wood WC, Barcos M, Henderson IC. c-erbB-2 expression and s-phase activity predict response to adjuvant therapy in women with node positive early breast cancer. New Engl. Jr. Med. 330: 1260-6, 1994

[18] Berry DA, Thor A, Cirrincione C, Edgerton S, Muss H, Marks J, Liu E, Wood W, Budman W., Perloff M, Peters W, Henderson IC (1995). Scientific Inference and Predictions; Multiplicities and Convincing Stories: A Case Study in Breast Cancer Therapy. In Bayesian Statistics 5. Oxford, Proceeding of the Fifth Valencia International Meeting June 5-9, 1994, 45-67. Oxford: Clarendon Press (Eds: Bernardo JM, Berger JO, Dawid AP, and Smith AFM) 1996

[19] Thor A, Muss HG, Berry DA, Dgerton SM, Kute T, Cirrincione CT, Budman DR, Wood WC, Barcos M, Koerner F, Henderson IC. p53 accumulation in State II breast cancers treated with adjuvant CAF. Results of CALGB 8869. In preparation.

[20] Barr L, Baum M., Time to abandon TNM staging of breast cancer? Lancet 339: 915-17 1992.

[21] Parent ME, Ghadirian P, Lacroix A, Perret C. Accuracy of reports of familial breast cancer in case-control series. Epidemiology 6:184-6, 1995

[22]Kornblith, AB, Holland JC. A model for quality of life research from the Cancer and Leukemia Group B: The telephone interview conceptual approach to measurement and theoretical framework. Journal of the National Cancer Institute Monograph 1996;20:55-62.

[23]Kornblith, A.B., Anderson, J., Cella, D.F., Tross, S., Zuckerman, E., Cherin, E., Henderson, E.S., Weiss, R.B., Cooper, M.R., Silver, R.T., Leone, L., Canellos, G.P., Gottlieb, A. & Holland, J.C. (1990). Quality of life assessment of Hodgkin's disease survivors: A model for cooperative clinical trials. Oncology, 4 (5), 93-101.

[24]Kornblith, AB, Anderson, J, Cella DF, Tross S, Zuckerman E, Cherin E. Henderson, ES Weiss, RB, Cooper MR, Silver RT, Leone L, Canellos GP, Gottlieb A, Holland JC. Hodgkin's disease survivors at increased risk for problems in psychosocial adaptation. Cancer 1992; 70:2214-2224.

[25]Greenberg DB, Herndon JE, Kornblith AB, Zuckerman E, Schiffer CA, Weiss RB, Mayer RJ, Wolchok SM, Holland JC. Long term psychosocial adaptation of survivors of acute leukemia. {Abstract}.Proc ASCO 1995; 14:No. 1668, p. 508.

[26]Kornblith, AB, Hollis DR, Zuckerman E, Lyss AP, Canellos GP, Cooper MR, Herndon JE, Phillips CA, Abrams J, Aisner J, Norton L, Henderson C, Holland JC. Effect of megestrol acetate upon quality of life in a dose-response trial in women with advanced breast cancer. Journal of Clinical Oncology 1993; 11: 2081-2089.

[27]Hubbard R, Lewontin RC. Pitfalls of genetic testing. edit. New Engl. Jr. Med 334: 1192-3, 1996.
[28] Wang, et al, Detection of Mammary Tumor Virus Env. Gene-Like Sequences in Human Breast Cancer. Cancer Research, 22:5173-5179, 1995

[29] Sancar A. Mechanisms of DNA excision repair. Science 266: 1954-56, 1994.

[30] Chaney S, Sancar A. DNA repair: enzymatic mechanisms and relevance to drug response. J Natl Cancer Inst 88: 1346-60, 1996

[31] Wei Q, Spitz M, Gu J, et al. DNA repair capacity correlates with mutagen sensitivity in lymphoblastoid cell lines. Cancer Epidem Biomarkers Prev 5: 199-204,1996

[32] Knight R, Parshad R, Price F, et al. X-ray-induced chromatid damage in relation to DNA repair and cancer incidence in family members. Int J Cancer 54: 589-93, 1993

[33] Scott D, Spreadborough A, Levine E, et al. Genetic predisposition in breast cancer. Lancet 344: 1444, 1994

[34] Helzlsouer K, Harris E, Parshad R, et al. Familial clustering of breast cancer: possible interactions between dna repair proficiency and radiation exposure in the development of breast cancer. Int J Cancer 64: 14-17, 1995

[35] Helzlsouer K, Harris E, Parshad R, et al. DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl Cancer Inst 88: 754-755 ,1996

[36] Parshad R, Price R, Bohr V, et al. Deficient DNA repair capacity, a predisposing factor in breast cancer. Br J Cancer 74: 1-5, 1996

[37]Callebaut I, Mornon J-P. From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair. FEBS lett 400: 25-30, 1997

[38] Scully R, Chen J, Plug A, et al. Association of BRCA1 with RAD51 in mitotic and meiotic cells. Cell 88: 265-75, 1997

[39] Sharan S, Morimatsu M, Albrecht U, et al. Embryonic lethality and radiation hypersensitivity mediated by RAD51 in mice lacking BRCA2. Nature 386: 804-810, 1997

FF Principal Investigator/Program Director (Last, first, middle):

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
BLEIWEISS, Ira Jay, M.D.	Associate Professor, Dept. of Pathology

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Univ. of Pennsylvania, Philadelphia, PA	B.S.	1980	Biological Basis of Behavior
St. George's Univ., School of Medicine Grenada, WI	M.D.	1984	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

Professional Experience

1997-pres.	Associate Attending, Dept. of Pathology, Mount Sinai Hospital, New York, NY
1997-pres.	Associate Professor, Dept. Of Pathology, Mount Sinai School of Medicine, New York,
1992-pres.	Associate Director, Laboratory Immunopathology, Division of Immunology, Mount Sinai Hospital, New York, NY
1993-1997	Assistant attending, Dept. of Pathology, Mount Sinai Hospital, New York, NY
1991-pres.	Assistant Professor, Dept. of Pathology, Mount Sinai School of Medicine, New York, NY
1990-1993	Clinical Assistant, Department of Pathology, Mount Sinai Hospital, New York, NY
1987-1989	Associate, Dept. of Pathology, Mount Sinai School of Medicine, New York, NY
1989-1991	Instructor, Dept. of Pathology, Mount Sinai School of Medicine, New York, NY

Publications

Bleiweiss IJ, Strauchen J: Lymphomatoid granulomatosis of the lung, report of a case and gene rearrangement studies. Hum Pathol 19:1109-1112, 1988.

Bleiweiss IJ, Jagirdar JS, Klein MJ, Siegel JL, Krellenstein DJ, Gribetz AR, Strauchen JA: Granulomatous pneumocystitis carinii pneumonia in 3 patients with the Acquired Immune Deficiency Syndrome. Chest 94:580-583, 1988.

Bleiweiss IJ, Dumitrescu OL, Jagirdar J: Angiofollicular lymphoid hyperplasia associated with myelofibrosis: Case report and immunological marker studies. Hemato Oncol 6:275-284, 1988.

Frydman C, Bleiweiss IJ, Yoo O, Jagirdar J: Diagnosis of cytomegalovirus infection of the lung in the Acquired Immune Deficiency Syndrome by in situ DNA hybridization. Prog in AIDS Pathol 1:91-100, 1989.

Bleiweiss IJ, Harpaz N, Wagner R, Biller HF: Functioning lipoadenoma of the parathyroid: A case report and literature review. Mt Sinai J Med 56:114-117, 1989.

Bleiweiss IJ, Schwartz IS, Mizrachy B, Kaneko M: Infiltrating cystic carcinoma of the breast: An unusual variant of breast cancer: A case report. Breast Dis 2:87-92, 1989.

Heller DS, Frydman CP, Klein MJ, Bleiweiss IJ, Bacall C: Luteoma of pregnancy. Mt Sinai J Med 57:40-42, 1990.

Lin J, Bleiweiss IJ, Mendelson MH, Szabo S, Schwartz IS: Cytomegalovirus-associated appendicitis in a patient with the Acquired Immune Deficiency Syndrome. Am J Med 89:377-379, 1990.

Bleiweiss IJ, Klein MJ: Chondromyxoid fibroma: Report of six cases with immunohistochemical studies. Modern Pathol 3:664-666, 1990.

Dicpinigaitis PV, Bleiweiss IJ, Krellenstein DJ, Halton KP, Teirstein AS: Primary endobronchial actinomycosis in association with foreign body aspiration. Chest 101:283-285, 1992.

Feltingoff M, Heller D, Bleiweiss IJ: Ovarian pregnancy with ipsilateral mature cystic teratoma of the ovary: Report of a case. Mt Sinai J Med 59:82-84, 1992.

Frydman CP, Bleiweiss IJ, Unger PD, Gordon RF, Brazenas NV: Identification of human papillomavirus subtypes in cervical biopsies using in situ DNA hybridization with biotinylated probes. J Reprod Med 37:151-156, 1992.

Brodman M, Dottino P, Friedman F, Heller D, Bleiweiss IJ, Sperling R: Treatment of vaginal and cervical human papillomavirus associated lesions with combination laser and topical 5-Fluorouracil. J Reprod Med 37:453-456, 1992.

Hermann G, Keller R, Tartter P, Bleiweiss IJ, Cohen J-M, Rabinowitz JG: Lobular Carcinoma in situ (LCIS) as a nonpalpable breast lesion: Mammographic and pathologic correlation. Breast Dis 6:269-276, 1993.

Copeland M, Kressel A, Spiera H, Hermann C, Bleiweiss IJ: Systemic inflammatory disorder related to fibrous breast capsules after silicone implant removal: Case report and review of the literature. Plast Reconst Surg 92:1179-1181, 1993.

Gold JE, Bleiweiss IJ, Goldfarb AB, Vauer JJ, Gelernt IM, Schwartz ME, Reiner MA, Miller CM, Weiss MF, Brower ST, Maters TR, OsbandME: Adoptive cellular therapy of human breast and colorectal tumor targets using ex vivo activated memory T-lymphocytes with potentiation by cis-diamminedichloroplatinum (II). J Surg Oncol 55:222-228, 1994.

Broiman SJ, Pan W, Pozner J, Weiss M, Bleiweiss IJ: Ductal adenocarcinoma arising in duodeno-pyloric heterotopic pancreas. Int J Surg Pathol 2(1):37-42, 1994.

Vine AJ, Bleiweiss IJ, Mizrachy B: Aeromonas hydrophila breast abscess. Breat Dis 7:387-391, 1994.

Copeland M, Choi M, Bleiweiss IJ: Silicone breakdown and capsular synovial metaplasia in textured-wall saline breast prostheses. Plast Reconst Surg 92:628-633, 1994.

Ahmed S, Tartter PI, Brower ST, Weiss SE, Brusco C, Bossolt K, Bleiweiss IJ, Amberson JB: Comparison of invasive cancers with and without extensive intraductal component. Breast Dis 8:1-6, 1995.

Hermann G, Keller RJ, Tartter P, Bleiweiss IJ, Rabinowitz JG: Interval changes in nonpalpable breast lesions as an indication of malignancy. Can Assoc Radiol J 46:105-110, 1995.

Jaffer S, Goldfarb AB, Gold JE, Szporn A, Bleiweiss IJ: Contralateral axillary lymph node metastasis as the first evidence of locally recurrent breast carcinoma. Cancer 75:2875-2878, 1995.

Seljo L, Sidhu J Mizrachy B, Shafir M, Tartter P, Bleiweiss IJ: Malignant phyllodes tumor of the breast: A report of 4 cases with associated fibroadenomata. Int J Surg Pathol 3:17-22, 1995.

Hoon V, Paradny R, Bleiweiss IJ: Mixed mammary and mullerian type glandular inclusions in an axillary lymph node: Case report and a review of literature. Breast Dis 8:363-368, 1995.

Brower ST, Tartter PI, Ahmed S, Brusco CM, Bossolt K, Hayden C, Bleiweiss IJ: Proliferative indices and oncoprotein expression in benign and malignant breast biopsies. Ann Surg Oncol 2:416-423, 1995.

Wang Y, Holland JF, Bleiweiss IJ, Melana S, Liu X, Pelisson I, Cantarella A, Stellrecht K, Mani S, Pogo BG-T. Detection of mammary tumor virus ENV gene-like sequences in human breast cancer: Human breast cancer and retroviral sequences. Cancer Res 55:5173-5179, 1995.

Garfein CF, Aulicino M, Leytin A, Drossman S, Hermann G, Bleiweiss IJ: Myoid hamartoma of the breast: A potential diagnostic pitfall for core biopsies. Arch Pathol Lab Med 120:676-680, 1996.

Tulchin N, Ornstein L, Bleiweiss IJ, Dikman S, Cardiff RD: Immunohistologic c-myc protein in benign breast disease and cancer. Int J Oncol 9:419-425, 1996.

Copeland M, Cooper B, Hermann G, Natarajian S, Unger PD, Bleiweiss IJ: Absent silicone shell in a MEMF polyurethane silicone breast implant: Report of a case and review of the literature. The Breast J 2:340-344, 1996.

Jing Y, Zhang J, Bleiweiss IJ, Waxman S, Zelent A, Mira-y-Lopez R: Defective expression of cellular retinol protein type I and retinoic acid receptors a2 62 and I2 in human breast cancer cells. The FASEB J 10:1064-1070, 1996.

Weston A, Pan C, Kaieski HB, Wallenstein S, Berkowitz GS, Tartter PI, Bleiweiss IJ, Brower ST, Senie RT, Wolff MS: p53 haplotype determination in breast cancer. Cancer, Epidemiol, Biomarkers, and Prevention 6:105-112, 1997.

Walsh MM, Drew M, Bleiweiss IJ: Neurofibroma of the common bile duct. A case report and review and review of the literature. Int J Surg Pathol, 4:245-248, 1997.

Bleiweiss IJ, Drossman S, Hermann G: Accuracy in mammographically directed breast biopsies: Communication is key. Editorial. Arch Pathol Lab Med, 121:357-358, 1997.

Kuerer HM, Bleiweiss IJ, Weiss MF: Bilateral Paget's disease of the male breast. In press, The Breast Journal, 1997.

Cunningham JD, Weiss SE, Ahmed S, Bratton JM, Bleiweiss IJ, Tartter PI, Brower ST: The efficacy of neoadjuvant chemotherapy compared to post-operative therapy in the treatment of locally advanced breast cancer. In press, Cancer Invest, 1997.

Tartter PI, Bleiweiss IJ, Levchenko S: Factors associated with clear biopsy margins and with clear re-excision margins in breast cancer specimen from patients desiring breast conservation. In press, J Am Coll Surgeons, 1997.

Aulicino MR, Dembitzer R, Szporn AH, Mechanick J, Batheja N, Bleiweiss IJ, Burstein DE: Cytologic findings in a case of C-cell hyperplasia. In press, Acta Cytologica, 1997.

Pogo BGT, Holland JF, Wang Y, Melana SM, Pelisson I, Liu B, Go V, Bleiweiss IJ: En busca de secuencias retrovirales relacionadas con el cancer de mama human. In press, Medicina (Spanish), 1997.